

11. (Amended) The vector of Claim 10 wherein, SEQ ID NO:3 or SEQ ID NO:4 is operably linked in a sense orientation with respect to said transcriptional initiation sequence.

12. (Amended) The transcriptional initiation sequence of Claim 9, wherein said initiation sequence provides wound induced expression of SEQ ID NO:3 or SEQ ID NO:4.

14. (Amended) A degenerate primer pair based on Phenylalanine ammonia-lyase homologous sequences in closely related plants, wherein first primer of said paired primers is GAYCCNYTNAAYTG GGG (SEQ ID NO:6) and second primer of said paired primers is CCYTGRAARTTNCCNCCRTG (SEQ ID NO:7).

REMARKS

Claims 1-14 are pending in this application. Claims 1, 2, 4, 6, 9, 11, 12 and 14 have been amended. The amendments to claims 1, 2, 4, 6, 9, 11 and 12 correct the assigned identifiers for SEQ ID NOS: designated in these claims. This renumbering of claim sequences was made necessary due to the misnumbering of assigned identifiers for amino acid sequences when referring to nucleotide sequences, and *vice versa*. The amendments to claim 14 insert sequence identifiers where required in order to conform to the Sequence Rules in adherence with 37 C.F.R. §§1.821 to 1.825.


Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-13, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification and Claims by the current Amendment. The attached pages are captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE."** As a convenience to the Examiner, a complete set of the Claims, as amended herein, is also attached to this Amendment as an Appendix entitled **"PENDING CLAIMS WITH ENTRY OF THE AMENDMENT."**

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,


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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 22 of page 3 has been amended as follows:

In another aspect, sequences are provided encoding *Lactuca sativa* PAL enzyme and obtainable by polymerase chain reaction of paired degenerate primers GAYCCNYTNAAAYTGGGG (SEQ ID NO:6) and CCYTGRAARTTNCCNCCRTG (SEQ ID NO:7).

Paragraph beginning at line 23 of page 4 has been amended as follows:

Figure 2: Comparison of the conserved regions of several known sequences to PAL (SEQ ID NOS:8-10), from sunflower (HA), *Arabidopsis* (AT), parsley (PC), carrot (DC), tobacco (NT), rice (OS) and wheat (TA), used to design primers for PCR (Genbank accession numbers in parenthesis).

Paragraph beginning at line 29 of page 4 has been amended as follows:

Figure 4: The cloning strategy adopted to obtain the full-length cDNA.
Conserved PAL peptides = SEQ ID NOS:11 and 9.

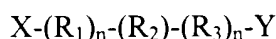
Paragraph beginning at line 20 of page 6 has been amended as follows:

Polypeptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising a sequence selected from the group of a sequence contained in Figure 5, and sequences encoding the amino acid sequence of

Figure 6 (SEQ ID NOS:3 and 1, respectively) (~~SEQ ID NOS: 1 and 2, respectively~~). The open reading frame begins at the ATG at base 119, and continues to the stop at 2254.

Paragraph beginning at line 10 of page 8 has been amended as follows:

The invention also includes polynucleotides of the formula:



wherein, at the 5' end, X is hydrogen, and at the 3' end, Y is hydrogen or a metal, R_1 and R_3 are any nucleic acid residue, n is an integer between 1 and 3000, preferably between 1 and 1000 and R_2 is a nucleic acid sequence of the invention, particularly a nucleic acid sequence selected from the sequence of SEQ ID NO:3 ~~SEQ ID NO: 1~~ and nucleic acid sequences encoding the peptide of SEQ ID NO:1 ~~SEQ ID NO: 2~~. In the formula, R_2 is oriented so that its 5' end residue is at the left, bound to R_1 , and its 3' end residue is at the right, bound to R_3 . Any stretch of nucleic acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

Paragraph beginning at line 21 of page 19 has been amended as follows:

Phenylalanine ammonia-lyase is highly conserved in plants, figure 2, therefore degenerate primers were developed to identify PAL genes in lettuce. As per Figure 2, degenerate primers were designed for polymerase chain reaction (PCR) based on peptide sequences which were similar among sunflower, *Arabidopsis*, parsley, carrot, tobacco, wheat and rice sequences. The peptide sequences chosen for PCR include a region near the 5' end of the PAL encoding sequence, peptide fragment DPLNW (SEQ ID NO:11), and a sequence approximately one-third from the 3' end, encoding the peptide fragment HGGNFQG (SEQ ID NO:9).

Paragraph beginning at line 28 of page 19 has been amended as follows:

The degenerate primers produced for PCR from these peptide fragments were GAYCCNYTNAAYTGGGG (5') (SEQ ID NO:6) and CCYTGRAARTTNCCNCCRTG (3') (SEQ ID NO:7). These primers were used to PCR amplify a portion of the open reading frame (ORF) from a *Lactuca sativa* cDNA library. The above primer pairs yielded PCR product which was in the expected range of 1.1 kb (Figure 3). The PCR products were then cloned into a vector which is amplified by expression of the cloned genes in bacteria. Bacterial colonies were selected and checked for the presence of vector insertions. DNA was then purified from the bacterial colonies.

Paragraph beginning at line 22 of page 20 has been amended as follows:

The methodology used for expression and purification of the fusion protein, MBP-PAL1, in *E. coli* was made following the procedures shown by Nonogaki et al. (2000) with differences explained as follow. Two primers were designed which complement the protein-encoded sequence of LsPAL1. The forward primer (5'-CGGAATTCATGGAGAACGGTAAT-3'; SEQ ID NO:12) included an EcorI site, while the reverse primer (5'-CGTCTAGACTAACATATTGGAAG-3'; SEQ ID NO:13) incorporated an XbaI site. The PAL open reading frame was cloned into the EcorI and XbaI site in pMALc vector (New England Labs, MN). The transformed bacteria were incubated overnight at 37 °C. An aliquot of the overnight culture was used to inoculate an incubation broth for 4 h at 37 °C. The cells were harvested by centrifugation and resuspended in sonication buffer (Nonogaki et al., 2000). After freezing overnight, the cells were thawed and sonicated for 5-10 min to release a higher amount of soluble fusion protein. The soluble protein was purified as Nonogaki et al. (2000) and separated by electrophoresis in a 10% acrylamide gel. The bands were stained with Coomassie brilliant blue (Fisher, PA) for approximately 1 h, and de-stained to visualize the major

bands. A pre-stained broad range protein standard (Bio-Rad, CA) was used to estimate the molecular weights.

In the Claims:

Claims 1, 2, 4, 6, 9, 11, 12 and 14 have been amended as follows:

1. (Amended) An isolated nucleic acid comprising a nucleotide sequence or a fragment thereof encoding the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 ~~SEQ ID NO:3~~.

2. (Amended) The nucleic acid of Claim 1, wherein said nucleotide sequence comprises the nucleotide sequence set forth in SEQ ID NO:3 ~~SEQ ID NO:2~~ or SEQ ID NO:4 or a fragment thereof of at least 18 base pairs up to the full length of the open reading frame encoding said amino acid sequence.

4. (Amended) A nucleic acid fragment that hybridizes to SEQ ID NO:3 ~~SEQ ID NO:2~~ or SEQ ID NO:4 under stringent hybridization conditions and has other than a nucleotide sequence as shown in Figure 2.

6. (Amended) An antibody that binds specifically to the amino acid sequence or portion thereof set forth in SEQ ID NO:1 or SEQ ID NO:2 ~~SEQ ID NO:3~~.

9. (Amended) An isolated nucleic acid construct comprising a transcriptional initiation sequence operably linked to SEQ ID NO:3 ~~SEQ NO:2~~ or SEQ ID NO:4 ~~SEQ NO:4~~.

11. (Amended) The vector of Claim 10 wherein, SEQ ID NO:3 ~~SEQ NO:2~~ or SEQ ID NO:4 ~~SEQ NO:4~~ is operably linked in a sense orientation with respect to said transcriptional initiation sequence.

12. (Amended) The transcriptional initiation sequence of Claim 9, wherein said initiation sequence provides wound induced expression of SEQ ID NO:3 ~~SEQ NO:2~~ or SEQ ID NO:4 ~~SEQ NO:4~~.

14. (Amended) A degenerate primer pair based on Phenylalanine ammonia-lyase homologous sequences in closely related plants, wherein first primer of said paired primers is GAYCCNYTNAAYTG GGG (SEQ ID NO:6) and second primer of said paired primers is CCYTGRAARTTNCCNCCRTG (SEQ ID NO:7).

PENDING CLAIMS WITH ENTRY OF THE AMENDMENT

1. (Amended) An isolated nucleic acid comprising a nucleotide sequence or a fragment thereof encoding the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

2. (Amended) The nucleic acid of Claim 1, wherein said nucleotide sequence comprises the nucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 or a fragment thereof of at least 18 base pairs up to the full length of the open reading frame encoding said amino acid sequence.

3. (As filed) The nucleic acid of Claim 2, wherein said fragment is between 18 and 500 base pairs.

4. (Amended) A nucleic acid fragment that hybridizes to SEQ ID NO:3 or SEQ ID NO:4 under stringent hybridization conditions and has other than a nucleotide sequence as shown in Figure 2.

5. (As filed) The nucleic acid fragment of Claim 4, wherein the fragment contains a label for detection selected from the group consisting of a radioisotope, an enzyme, a particle and a protein.

6. (Amended) An antibody that binds specifically to the amino acid sequence or portion thereof set forth in SEQ ID NO:1 or SEQ ID NO:2.

7. (As filed) The antibody of Claim 6 wherein said antibody is polyclonal.

8. (As filed) The antibody of Claim 7 wherein said antibody is monoclonal.

9. (Amended) An isolated nucleic acid construct comprising a transcriptional initiation sequence operably linked to SEQ ID NO:3 or SEQ ID NO:4.

10. (As filed) A recombinant vector comprising the nucleic acid construct of Claim 9.

11. (Amended) The vector of Claim 10 wherein, SEQ ID NO:3 or SEQ ID NO:4 is operably linked in a sense orientation with respect to said transcriptional initiation sequence.

12. (Amended) The transcriptional initiation sequence of Claim 9, wherein said initiation sequence provides wound induced expression of SEQ ID NO:3 or SEQ ID NO:4.

13. (As filed) A transgenic plant cell or bacterial cell comprising the vector of Claim 11.

14. (Amended) A degenerate primer pair based on Phenylalanine ammonia-lyase homologous sequences in closely related plants, wherein first primer of said paired primers is GAYCCNYTNAAYTGGGG (SEQ ID NO:6) and second primer of said paired primers is CCYTGRAARTTNCCNCCRTG (SEQ ID NO:7).